STUDIES OF THE EFFECTS OF EXTRACTS FROM TWO LOCAL PLANTS (TEPHROSIA VOGELII AND PARKIA CLAPPERTONIANA) ON CLARIAS GARIEPINUS (TENGELS).

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ABSTRACT

Studies were carried out using 96hr static toxicity bioassay to determine the effect of lethal concentrations of extracts from two local plants Tephrosia vogelii and Parkia clapper toniana, which are known fish poison, on a species of mud fish, Clarias gariepinus. Phytochemical analysis of the plant extracts was done and the extract from T. vogelii was found to contain alkaloids, tannins and flavonoids while the extract from P. clappertoniana was formed to contain alkaloids, tannins and saponins. Experimental fish were exposed to test water separately polluted bu varying concentrations of extraction of both plant species ranging from 0.50mgl^{-1}, 1.50 mgl^{-1}, 2.50 mgl^{-1}, 3.0 mgl^{-1}, 5.00 mgl^{-1}, 10.00 mgl^{-1}, in the case of T. vogelii and 5.00 mgl^{-1}, 7.50 mgl^{-1}, 10.00 mgl^{-1}, 15.00 mgl^{-1}, 20.00 mgl^{-1} and 30.00 mgl^{-1} in the case of P. clappertaniana. Behavioural hispathological and heamatological examinations were made. Both plant extracts were found to have lethal effects at the higher concentrations, affecting the gills and the central nervous system as well as having a depressive effect on the total count and increasing platelet and white blood cell count. Symptoms of toxicosis observed include, initial inactivation agitated swimming, tumbling movement air gulping, increased opercular beat and period of quiescence/knockdown before death. Marked differences were also observed in the hematological and histopathological analysis of poisoned fish. Lower concentrations of the extracts had sub lethal effects on the fish which manifested as zig-zag movement, air gulping, increased opercular movement etc. None of these effects were observed in the control experiment.

INTRODUCTION

Certain plants contain or produce substances capable of harming or killing animals both on land and in water. Plant poisons can be classified bacterially, chemically or according to their physiological activity. There are five main groups into which plant poisons can be classified with respect to their physiological activity. These are irritants, surface/contact poisons, blood poisons, muscular poisons and nerve poisons. Phytochemical analysis of plants containing poisons has shown that they contain diverse toxic substances. Schuttes (1970) mentions these as including alkaloids, saponines, cyanogenic substance, solanine, glycosides, cesinoids, volatine oil, tannins, oxalic acid, phytotoxine/toxalbumins and selenium. Plants containing many of these substances are used in many parts of the world as a simple and cheap means of obtaining fish from small water bodies. Reed et al (1967) reported some of the tribes of northern Nigeria that have been using plant poisons to catch fish from time immemorial and mentioned some of the commonest plants used for the purpose to include Tephrosia vogelii, Parkia clappertoniana, Mundulea sericea, Lasiosiphona krussianus, Boerhaavia coccinea and Acacia pennata.

Reed et al (1967) reported that T. vogelii is particularly cultivated on forms for the purpose of being used to kill fish. Gill (1992) reports that the plant T. vogelii contains Deguelin and Tephrosin in the leaves, roots, seeds and fruit capsules as well as Toxiconol, Tephrosal and quercitin Reed et al (1967) reported that the principal active ingidients in T. vogelii is Tephrosin ($C_{23}H_{22}O_7$) which is closely related both chemically and in action to the commercial insecticide and fish poison Rotenone ($C_{23}H_{22}O_6$) used in killing undesirable fish. Irvine (1961) reported that

the toxin in T. vogelii is Tephrosin and that the highest concentration is in the leaves (15%) followed by the seeds (3%) and them other parts. Freyre and Barned (1967) found that fresh leave extracts were more effective in their pisicidal activity than oven dried leaves Donold and Ruben (1968) reported that leaves nearest to the apex and around the periphery of the plant showed a greater accumulation of Tephrosin and the older leave nearest the main stem showed a marked reduction in Tephrosin content.

Parkia clappertoniana is commonly found growing in the wild across the sub-region. Ademoroti (1996) reports that P. Clappertoniana contains tannins, and alkaloids while Adam (1966) reported the presence of alkaloid, saponins and tannins in the bark of P. Clappertoniana. The husk was found to contain a higher amount of tannins (27.3%) followed by lesser quantities of alkaloids and saponins.

Both of these plants are collected and pounded to make fish poisons, the full mature pods after the pulp and seed have been extracted for food (Osibanjo, 1991) in the case of P. Clappertoniana and the shoot in the case of T. vogelii. These are used to pollute natural waters and when the chemical contaminants reach levels in excess of the assimilative capacity of the receiving water, the survival, reproduction, growth and movement of organisms in such waters can be affected (Lawee, 1997). There is no doubt that these plant poisons are very effective in killing fish since fish are extremely sensitive to aquatic pollution (Sprague, 1970).

Lethal and sub lethal concentrations of plant poisons are known to have toxic effects on fish behaviour, heamatology, histopathology, growth, reproduction, feeding, respiration and general other physiological processes of exposed organisms. (Butler 1971).

The objective of this study therefore is to study the effects of lethal and sublethal doses of extracts from T. vogelii and P. clappertoniana on juveniles of a hardly, culturable fish species, Clarias gariepinus commonly found in this locality and which is actively fished using these poisonous plants materials.

MATERIALS AND METHODS

Fresh leaves of Tephrosia vogelii from the apex of the plant were collected from the locality at mid-day. The husk of the pod of P. clappertoniana were also collected from the locality and pounded and ground separately to powder form. A cold extraction method after Kavanagh (1983) was used for the extraction and the extract was lyophylised using a deep freezer. Phytochemical analysis were carried out on the extract using ethanoic KOH, $FeCl_3$ reagent, Fehlines solution A&B and Dragendorffs reagent to test for the presence of flavonoids, tannins, saponins and alkaloids respectively.

Healthy, active juveniles of Clarias gariepinus were obtained from a local fish farm and used for the study. Fish used in the study had an average standard length 20.00±1.50 and average weight of 110±2.0g.

Following the methods of Sprague (1970) and APHA(1985); static 96hr toxicity bioassay were carried out in the laboratory. Experimental fish were exposed to test water separately polluted with varying concentrations of extracts of both plant species ranging from 0.50 mgl^{-1}, 1.50 mgl^{-1}, 3.50 mgl^{-1}, 5.00 mgl^{-1} and 10.00 mgl^{-1} in the case of T. vogelii to 5.00 mgl^{-1}, 7.50 mgl^{-1}, 10.00 mgl^{-1}, 15.00 mgl^{-1}, 20.00 mgl^{-1} and 30.00 mgl^{-1} as the case of P. clappertoniana based on the result of previous tests. The fishes were acclimatized at 25.00 – 26.5°c for two days in dechlorinated municipal water prior to experimentation. During acclimatization the fishes were fed twice daily at 5% body weight with pelleted feed obtained from Feed Masters Jebba road Ilorin. There was a control for each set of experiments in which the fishes were only exposed to dechlorinated municipal water. Each experiment was duplicated. The desired weight of lyophilized extract was added 40L of dechlorinated municipal water allowed to stand for 30 minutes before the introduction of test fishes. Fish were stocked at this rate of 10 fish per treatment tank. Physiochemical parameters of test water were examined using standard methods

as contained in APHA (1985), blood samples were taken and subject to haematological analysis at the UITH Ilorin while sections of selected tissues from tissues of fishes from all the concentrations tested and from both dead and live fishes were subjected to histopatological analysis at the UITH Ilorin. Behavioral and morphological changes were examined by observation. Result were subjected to analysis of variance (ANOVA) statistical test for differences between levels of treatment.

Results

The phytochemical analysis of the plant extracts showed that T. vogelii contained flavonoids, tannins and alkaloids while P. clappertoniana contained alkaloids, tannins and saponins (Table 1).

TABLE 1: PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACTS

Test	Observation	Inference
Alkaloids	T. vogelii	Alkaloids present
500mg of each extract on 1% HCl in steam bath + few drops of Dragendorffs reagent	Brown observed P. clappertoniana turns turbid	Alkaloids present
Flavonoids	T. vogelii	
200mg of each extract in 5ml of ethanol. Filtrate + few drops of ethanolic KOH	Clear yellow solution formed and persists on heating. P. clappertoniana No observable change	Flavonoids confirmed Flavonoids absent
Saponins	T. vogelii	
250mg of each extract shaken with 10ml distilled water until fronting and heated at 59°c for 45 mins in water bath or 1ml of filtrate can be treated with few drops of Fehlins solution A&B	No visible reaction observed. P. clappertoniana fronting continues even after heating	Saponins absent Saponins present
Tannins	T. vogelii	
500mg of each extract dissolved in 10ml of distilled water + few drops of ferric chloride reagent	Blue black collaboration observed P. clappertoniana blue black coloration observed	Tannins present Tannins present

TABLE 2: PHYSIOCHEMICAL ANALYSIS OF TEST WATER

	T. vogelii						P. clappertoniana					
Parameter	0.5mg	1.50mg	2.5	3.50	5.00	10.00	5.00	7.50	10.00	15.00	20.00	30.00
PH	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.8	6.9	6.9	6.8	6.8
Total Hardness (mg/l)	56.4	56.6	56.0	56.9	56.0	56.2	56.6	57.0	57.6	57.4	58.4	59.0
Dissolved oxygen (mg/l)	3.2	2.9	2.6	2.0	1.9	1.7	2.2	2.0	1.8	1.6	1.4	1.0
Temperature (^{0}c)	26.0	26.2	26.5	28.0	28.5	29.0	25.5	26.0	27.0	27.5	27.8	28.5
Colour change	-	-	-	-	-	-	-	-	Light brown	Light brown	Brown	Dark brown

Similar changes in behaviour of fish were observed in experiments involving both plant extracts. The changes in order of appearance include, initial inactivity of fish immediately after introduction into test water, with mouth closed and opecular action stopped lasting for just a few minutes, followed by fast agitated swimming action, then knockdown stage when they lie on their sides at the bottom followed by slow vertical swimming towards the surface, then air gulping at the surface while still in the vertical position, occasionally going down in the water and raising up again in the vertical position, then in the higher concentrations, fast agitated swimming with the head moving from side to side and somersaulting movements, then knock down to the bottom with no sign of opercular or muscular movement and then death. Death was confirmed when there was no response to gentle prodding with a stirring rod. In lower sub lethal concentrations after the vertical swimming stage at the surface, the fish returned to the normal horizontal swimming position and continued normal activity. It was generally observed that concentrations had an inversely proportional relationship with time of exposure of fish as highest concentrations had the shortest duration of action and the lowest concentration when fish survived, the highest period of exposure. A relatively constant behaviour of fish was observed in the control experiment throughout the duration of the experiment. With respect to the physicochemical analysis of the test water. It was observed that for P. clappertoniana pH, alkalinity and total harness values increased slightly with increase in concentrations. No significant difference was observed for these parameters in tests involving T. vogelii. For both plant extracts it was observed that concentration of extracts was inversely proportional to level of dissolved oxygen Dissolved oxygen reduced with increase in temperature and concentration on extract and temperature was found to increase with increase in concentration of extract(Table 2). In the treatment with P. Clappertoniana, an oil film developed on the surface of the water in the higher concentration after sometime. The higher the concentration the faster the oil film developed. This turned the water murky.

With respect to morphological changes, it was observed that for both plant extracts. dead fish from the higher concentrations showed as increase in standard length and weight after death and fish that remained alive, showed a decrease in standard length and weight after exposure (Table 3).

TABLE 3: MORPHOLOGICAL CHANGES OBSERVED IN EXPOSED FISH

	Tephrosia vogeli				
	AV. Standard length	AV. Standard length	AV. Weight	AV. Weight	Condition
Concentrates	Before treatment	After treatment	Before treatment	After treatment	
0.0mg	20.00	20.00	125	125	Live
0.50mg	20.00	20.00	110	110	Live
1.50mg	20.00	20.00	110	105	Live
2.50mg	21.50	21.50	125	110	Live
3.50mg	20.50	21.50	130	135	Dead
5.00mg	18.50	19.00	90	97	Dead
10.00mg	20.00	21.00	105	110	Dead

	PARKIA CLAPPERTONIANA				
Concentrations	AV. Standard length	AV. Standard length	AV. Weight	AV. Weight	Condition
	Before treatment	After treatment	Before treatment	After treatment	

0.0mg	20.50	20.50	105	105	Live
5.00mg	21.00	20.40	100	90.5	Live
7.50mg	21.50	20.80	110	105	Live
10.00mg	21.50	20.30	136	130	Live
15.00mg	19.50	19.70	105	108	Dead
20.00mg	20.00	20.20	100	104	Dead
30.00mg	21.00	21.00	130	135	Dead

With respect to heamatological studies, it was observed that with increase in concentration there was a decrease in heamoglobin (Hb) level, packed cell volume(PCV), mean corpuscular heamoglobin (MCH), mean corpuscular volume(MCV), RBC and mean corpuscular heamoglobin count (MCHC). There was a decrease in lymphocytes count but an increase in WBC and neutrophil count. (Table 3) Microscopic examination of blood pressure showed that the rod blood cells of some exposed fish had abnormal sizes in which some were smaller, some bigger and some were of abnormal shape.

Histopathological examination of exposed fish showed distortion of epithelial cells of the gills in the higher concentrations of both plant extracts. The higher the concentration, the greater the degree of distortion until complete necrosis of epithelial cells occurred. The structure of the gonads was also distorted with complete necrosis of gonads cells taking place at the higher concentrations. The liver also showed distortions with increase in concentration with the central canals, developing macrophages and the interstitial spaces of the body tissues increased with an increase in concentration of extract due to fusion of cuboidal cells.

DISCUSSION

Results of the photochemical analysis show that the plant extracts contain toxicants such as alkaloids which have stimulating effects on the gills thus stimulating increased opercula beat and may inpair respiration and osmo-regulation (Wrong et al 1971, Annune et al 1991). Reed et al (1967) reported that plants poisons affect the gills such that fish cannot breath and Boyd (1982) reported that rotenone interferes with regulation and is extremely toxicant fish in low concentrations. The initial inactivity of the fish on introduction into the test water and the closing of the month and storage of opercular movement may be attributed to avoidance behaviour. Gbem et al (1999) reported similar findings in fish. The knockdown stage may be as a result of loss of muscular control. Girah (1997) reports that difficulty in movement by fish could be due to the interference of the poison with the normal functioning of the nervous system and consequently the coordination of muscular activities, hence the vertical swimming action.

The steady deplation of total hardness in test water with increase in concentration causes the water to become increasingly harder thus reducing the amount of available dissolved calcium and magnesium ions present in the water. This reduces muscular activity in exposed fish, as the two ions serve as systematic electrolytes which naturally and muscular activity within fish (Farmer 1980). That dissolved oxygen decreased with increase in temperature may be due to the fact that dissolved gases generally decrease in solubility with increasing temperature which later effect the properties of aquatic environment (Hill 1976 and Robert 1978).

The increase in standard length and weight of dead fish may be due to muscular relaxation after death; while decrease in weight of live fish in the lower concentrations may be attributed to the stress the fish undergo while adjusting to attain a tolerance level with the pollutants. Auta et al (2002) also reported abnormal behaviours in fish exposed to lethal concentrations of toxicants.

Results of the haematological analysis shows that the poisons have a pronounced depressive effect on the blood forming system of the fish as shown by the decrease in values of

most of the parameters examined as the poison concentration increased. The results simply indicate a situation of anaemia. Robert (1978) reports that such anaemia may be due to a resultant deficiency in oxygen transportation. The increase in WBC and lymphocytes may be a possible indication of infection because the immune system of the fish has been sensitized by the poison and therefore buildings up more WBC to combat the effect of the poison. The platelet count also increased in direct proportion with the poison concentration. This could favour a situation of increased intravenous blood coagulability or clotting.

The distortions and necrosis of epithelial cells of the gills observed may be due to the direct exposure of the gills to the poison. This implies that they are liable to damage by any irritant material whether dissolved or suspended in water. The effect on the gonads and the macrophages seen in the central canal of the liver may be attributed to the effect of the plant poisons which gets to them through the blood circulating from the gills and affect their osmo-regulatory function. The increase in the interstitial spaces may be due to the accumulations of tissue fluids, which cause the cuboidal cells of the body tissues to fuse up.

CONCLUSION

From the foregoing it can be concluded both T. vogelii and P. clappertoniana are toxic plants which act as a respiratory poison at low concentrations affecting the gills and impairing respiration and osmo-regulation and having a depressive effect on the blood forming system of fish but act on the respiratory and nervous systems at high concentrations resulting in various abnormal behaviours and eventually death. It can also be concludede that T. vogelii is the more deadly of the two plant poisons.

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REFERENCES

Adam, S.I (1966): Phytochemical and Microbiological Investigation of Nigerian plants used in the treatment of skin disease. Publ., Obafemi Awolowo University, Ile-Ife Nigeria Pp 84-120

Ademoroti, C.M.A(1996): Environmental Chemistry and Toxicology publ. Foludex press Ltd Ibadan pp. 214-220.

Annune, PA, Gbele S.O and Oladimeji A.A (1991). Acute Toxicity of Zinc to fingerlings of Clarias lazera. J. Aquatic sciences 3:1357-1365.

APHA, (1985): American Public Health Association, American water worker Association water pollution control federation. Standard Methods for Examination of Water and Waste Waters (16th ed) APHA New York. 1193.

Auta, J. Balogun J.K, Lawal F.A and Ipinjolu, J.K (2002): Short-term effects of dimethoate on behaviour of juveniles of Oreochromis nicoticus (Trewavas) and Clarias gariepinus (tengels) Journal of Tropical bio-science Vol. 210:55-59 2002.

Butler, P.A (1971): Influence of Pesticides on Marine Ecosystems Proc. Royal. Soc London 177:321-329.

Donold, K.B and Ruban H.F(1968) Recovery of Natural insecticides from Tephrosia vogelii III. An improved procedure of sampling and assaying Rotenoid content in leaves J of economic Botany Vol. 20(5) 93-97.

Farmer, J.N (1980): Toxic Responses: Encyclopedia of Science and Technology Publ. McGraw-Hill. Vol 14. pp. 11-14.

Freyre, R H and Barned, D.K(1966) Recovery of Natural Insecticide from Tephrosia vogelii Efficiency of rotenoid extraction from each and oven dried leaver. J. of Economic Botany vol. 20(3) 279-284

Gbem, T.T, Balogun. F.A, Lawal, F.A and Annune, P.A(1999) Some Aspects of the acute toxicity of tannery effluent to juvenile Clarias gariepinus (Tengels).

Gills, L.S (1992): Ethanomedical uses of Plants in Nigeria publ. Macmillan Press Ltd. Pp229.

Girah, T.J (1992) cute toxicity of Rogor on Oreochromis niloticus. B.sc Thesis ABU Zaria (unpublished).

Hill, R.W(1976) Comparative Physiology of Animals in Environmental Approach. Publ. Howper & Row. Mc pp. 273.

Irvine, F.R(1961): Woody Plants of Ghana publ. Oxford University Press. Oo 415-416.

Lawee, A.U(1997): Acute Toxicity of Rogor on Clarias gariepinus. B.Sc Thesis ABU Zaria (unpublished).

Osibanjo, O (1991): FEPA guideline and standards for environmental pollutions and control. The way out. 21st August 1991. Lagos Nigeria.

Reed. W. Burchard J, Hopson, A.J Jenness J and Yaro I. (1967). Fish and Fisheries of Northern Nigeria publ. Ministry of Agriculture press Northern Nigeria. Pp. 201-202.

Robert R J (1978). Fish Pathology publ. Cassel Ltd London pp. 1-77

Sprague, J.B (1970). Measurement of Pollutant Toxicity of the Fish II utilizing and applying bioassay results. Water Research 4:3-32.

Wong. M H, Luke K.C and Choi, K.G (1999). Cypinus Carpio and Cteriopheryingodon Idellus Acta 99: 450-454.